

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of claims

1. (Currently Amended) A method of *in vitro* cultivation of *Polygonatum* comprising the steps of:

contacting a *Polygonatum* seed with a first medium comprising a MS basal culture medium and gibberellic acid (GA₃) present in an amount from about 10 mg/L to about 100 mg/L ~~gibberellic acid (GA₃);~~

upon emergence of a hypocotyl of a seedling, contacting ~~this primary explant~~ said seedling with a second medium comprising a MS basal culture medium, 6-benzyl-aminopurine present in an amount from about 3 mg/L to about 6 mg/L ~~of 6-benzyl-aminopurine~~, and naphthalene acetic acid (NAA) present in an amount from 0.5 mg/L to about 1.0 mg/L ~~naphthalene acetic acid; and~~

upon emergence of a first foliage leaf of said seedling, contacting ~~this secondary explant~~ said seedling with a third medium comprising a MS basal culture medium, 6-benzyl-aminopurine present in an amount of about 2.0 mg/L ~~6-benzyl-aminopurine~~, naphthalene acetic acid present in an amount of about 1.0 mg/L ~~naphthalene acetic acid~~, and GA₃ present in an amount from about 5 mg/L to about 20 mg/L ~~gibberellic acid (GA₃).~~

2. (Original) The method of claim 1, wherein the *Polygonatum* is selected from the group consisting of *Polygonatum cirrhifolium*, *Polygonatum oppositifolium*, and *Polygonatum verticillatum* L.

3. (Original) The method of claim 2, wherein the *Polygonatum cirrhifolium*, is *Polygonatum cirrhifolium* Royle.

4. (Original) The method of claim 2, wherein the *Polygonatum oppositifolium*, is *Polygonatum oppositifolium* Royle.

5. (Currently Amended) The method of claim 1, wherein ~~the concentration of~~ GA₃ in the first ~~media~~ medium is present in an amount from about 50 mg/L to about 100 mg/L.

6. (Currently Amended) The method of claim 1, wherein ~~the concentration of~~ NAA in the second ~~media~~ medium is present in an amount of about 1.0 mg/L.

7. (Currently Amended) The method of claim 1, wherein ~~the concentration of~~ GA₃ in the third ~~media~~ medium is present in an amount from about 15 mg/L to about 20 mg/L GA₃.

8. (Original) The method of claim 1, wherein the MS basal culture medium of the first, second, and/or third medium is MS basal culture medium I.

9. (Currently Amended) The method of claim 1, wherein the MS basal culture medium of the first, second, and/or third ~~media~~ medium is MS basal culture medium II.

10. (Currently Amended) The method of claim 1, wherein the first, second, and/or third ~~media~~ medium further comprise comprises agar.

11. (Original) The method of claim 1, wherein the MS basal culture medium of the first, second, and/or third medium has a pH of about 5.8.

12. (Currently Amended) The method of claim 1, wherein said contacting steps are performed at a ~~the~~ temperature is from about 20° C to about 24° C.

13. (Currently Amended) The method of claim 1, wherein said contacting steps are performed at a ~~the~~ relative humidity is about 50% to about 60%.

14. (Currently Amended) The method of claim 1, wherein said *Polygonatum* is contacted with said first medium in the ~~substantial~~ absence of light.

15. (Original) The method of claim 1, wherein said *Polygonatum* is contacted with said second and/or third medium under illumination of about 2000 to about 3500 lux.

16. (Original) The method of claim 1, wherein the primary explant is contacted with the second medium less than 60 days after said seeds are contacted with the first medium.

17. (Currently Amended) A method of *in vitro* cultivation of *Polygonatum cirrhifolium* Royle comprising the steps of

contacting a *Polygonatum cirrhifolium* Royle seed with a first medium comprising a MS basal culture medium and gibberellic acid (GA₃) present in an amount from about 10 mg/L to about 100 mg/L ~~gibberellic acid (GA₃)~~;

upon emergence of a hypocotyl of a seedling, contacting ~~this primary explant~~ said seedling with a second medium comprising a MS basal culture medium, 6-benzyl-aminopurine present in an amount from about 3 mg/L to about 6 mg/L ~~of 6-benzyl-aminopurine~~, and naphthalene acetic acid (NAA) present in an amount from 0.5 mg/L to about 1.0 mg/L ~~naphthalene-acetic acid~~; and

upon emergence of a first foliage leaf of said seedling, contacting ~~this secondary explant~~ said seedling with a third medium comprising a MS basal culture medium, 6-benzyl-aminopurine present in an amount about 2.0 mg/L ~~6-benzyl-aminopurine~~, NAA present in an amount about 1.0 mg/L ~~naphthalene-acetic acid~~, and GA₃ present in an amount from about 5 mg/L to about 20 mg/L ~~gibberellic acid (GA₃)~~.

wherein the steps are performed at a temperature is from about 20°C to about 24°C, and
the relative humidity is about 50% to about 60%,

wherein the hypocotyl-bearing seed is contacted with the second medium is contacted
with the seedling less than 60 days after said seeds are contacted with the first medium, and the
seedling is contacted with the third medium less than 90 days after said seeds are contacted with
the first medium.

18. (Currently Amended) A method of germinating ~~65-100% of *Polygonatum* seeds in~~
~~less than 60 days~~ comprising contacting a plurality of *Polygonatum* seeds with a first medium
comprising a MS basal culture medium and gibberellic acid (GA₃) present in an amount from
about 10 mg/L to about 100 mg/L ~~gibberellic acid (GA₃)~~

wherein 65-100% of the seeds are germinated and

wherein germination occurs in less than 60 days.

19. (Currently Amended) The method of claim 12, wherein ~~the germination rate is about~~
~~98% and the concentration of gibberellic acid (GA₃) is~~ GA₃ in said first medium is present in an
amount of about 50 mg/L, and

wherein 98% of the seeds generate a seedling.

20. (Currently Amended) A method of germinating *Polygonatum* seeds comprising
contacting a plurality of *Polygonatum* seeds with a first medium comprising a MS basal culture
medium and gibberellic acid (GA₃) present in an amount from about 50 mg/L ~~gibberellic acid~~
~~(GA₃)~~ wherein 65-100% of said *Polygonatum* seeds germinate in less than 60 days.

21. (Currently Amended) The method of claim 14, wherein ~~the germination rate is about 98% and the concentration of gibberellic acid (GA₃) is~~ GA₃ in said first medium is present in an amount of about 50 mg/L and wherein 98% of the seeds generate a seedling.

22. (Currently Amended) A method of inducing synchronous release of epicotyl dormancy, coleoptile dormancy, and radicle dormancy in *Polygonatum* comprising contacting a hypocotyl-bearing *Polygonatum* seed with a medium comprising a MS basal culture medium, 6-benzyl-aminopurine present in an amount from about 3 mg/L to about 6 mg/L ~~of 6-benzyl-aminopurine~~, and naphthalene acetic acid present in an amount from 0.5 mg/L to about 1.0 mg/L ~~naphthalene acetic acid~~.

23. (Withdrawn) A kit comprising a package insert and

- a first medium comprising MS basal culture medium and from about 10 mg/L to about 100 mg/L Gibberellic acid (GA₃);
- a second medium comprising from about 3 mg/L to about 6 mg/L of 6- benzyl-aminopurine (BAP), and from 0.5 mg/L to about 1.0 mg/L naphthalene acetic acid ; and
- a third medium comprising about 2.0 mg/L BAP, about 1.0 mg/L NAA, and from about 5 mg/L to about 20 mg/L GA₃.

24. (Withdrawn) A kit comprising a package insert and

- a germination medium comprising MS basal culture medium and from about 10 mg/L to about 100 mg/L Gibberellic acid (GA₃);
- a leafing medium comprising from about 3 mg/L to about 6 mg/L of 6- benzyl-aminopurine (BAP), and from 0.5 mg/L to about 1.0 mg/L naphthalene acetic acid ; and

a budding medium comprising about 2.0 mg/L BAP, about 1.0 mg/L NAA, and from about 5 mg/L to about 20 mg/L GA₃.

25. (Withdrawn) Culture media compositions useful for initiating extraordinarily fast and synchronized *in vitro* induction of germination and release of epicotyl dormancy in *Polygonatum cirrhifolium* Royle, an endangered medicinal plant species, said composition comprising modified Murashige and Skoog (MS) basal culture medium, varied concentrations of plant hormones, and other additives, said compositions are;

a. first medium composition useful for initiating fast germination of said plant species with percentage germination ranging between 65 to 98%, and duration period to achieve the same ranging between 7 to 53 days, and also useful for initiating fast release of epicotyl, coleoptile and radicle in percentage seeds ranging between 78 to 100, with duration period for synchronization of the same ranging between 11 to 18 days, said composition consisting of MS basal culture medium, Gibberellic acid (GA₃) ranging between 10 to 100 mg/L,

b. second medium composition useful for initiating fast release of first foliage leaf from epicotyl dormant explants, with percentage secondary explants producing leaves ranging between 65 to 86%, mean number of leaves per secondary explant ranging between 2.7 to 4, and mean leaf length ranging between 3 to 6 cms, said composition consisting of MS basal culture medium, 6- benzyl-aminopurine (BAP) ranging between 3 to 6 mg/L, and Naphthalene acetic acid (NAA) ranging between 0.5 to 1.0 mg/L, and

c. third medium composition useful for initiating fast release of axillary buds from epicotyl dormant explants, with secondary explants producing axillary buds ranging between 70 to 100%, mean number of axillary buds per secondary explant ranging between 6 to 12, and

axillary buds producing leaves ranging between 45 to 98%, said composition consisting of MS basal culture medium, 6- benzyl-aminopurine (BAP) about 2.0 mg/L, Naphthalene acetic acid (NAA) about 1.0 mg/L, and Gibberellic acid (GA₃) ranging between 5 to 20 mg/L,

wherein, above-mentioned first medium, second medium, and third medium are used in the same sequence for initiating extraordinarily fast and synchronized *in vitro* induction of germination and release of epicotyl dormancy in plant *Polygonatum cirrhifolium* Royle.

26. (Withdrawn) Compositions as claimed in claim 25 wherein, plant hormones are selected a from group comprising Gibberellic acid (GA₃), alpha- naphthalene acetic acid (NAA), and 6-benzyl-aminopurine (BAP).

27. (Withdrawn) Compositions as claimed in claim 25 wherein, Murashige and Skoog's (MS) basal culture medium is preferably consisting of 2.2g/L NH₄NO₃, 2.0g/L KNO₃, 0.44 g/ l CaCl₂. 2H₂O, 0.37 g/L MgSO₄. 7H₂O, 0.17g/L KH₂PO₄, 37.25 mg/L Na₂ EDTA, 27.8 mg/L FeSO₄.7H₂O, 0.83 mg/L KI, 6.2 mg/L H₃ BO₃, 22.3 mg/L MnSO₄. 4H₂O, 8.6 mg/L ZnSO₄. 7H₂O, 0.25 mg/L Na₂ MoO₄. 2H₂O, 0.025 mg/L CuSO₄. 5H₂O, 0.025 mg/L CoCl₂.6H₂O, supplemented with 250 mg/L myo-inositol, 0.5mg/l nicotinic acid, 0.5 mg/L pyridoxine HCl, 3 mg/L thiamine HCl, 2 mg/L glycine, and 30g/L sucrose.

28. (Withdrawn) Compositions as claimed in claim 25 wherein, first medium composition is used for fast germination, with percentage induction of germination of about 98% is achieved in time duration ranging between 7 to 23 days, said composition consisting of MS basal culture medium and about 50 mg/L Gibberellic acid (GA₃).

29. (Withdrawn) Compositions as claimed in claim 25 wherein, first medium composition is used for breaking epicotyl dormancy, with about 100% release of epicotyl,

coleoptile and radicle, and to achieve the same in time duration ranging between 11 to 14 days, said composition consisting of MS basal culture medium and Gibberellic acid (GA₃) ranging between 50 to 100 mg/L.

30. (Withdrawn) Compositions as claimed in claim 25 wherein, second medium composition is used for initiating fast release of first foliage leaf from epicotyl dormant explants, with about 86% secondary explants producing leaves, about 3.4 mean number of leaves per secondary explant, and about 6 cms mean leaf length, said composition consisting of MS basal culture medium, 6- benzyl-aminopurine (BAP) ranging between 3 to 6 mg/L, and Naphthalene acetic acid (NAA) about 1.0 mg/L.

31. (Withdrawn) Compositions as claimed in claim 25 wherein, third medium composition used for initiating fast release of axillary buds from epicotyl dormant explants, with about 100%, secondary explants producing axillary buds, number of axillary buds per secondary explant ranging between 9 to 12, and axillary buds producing leaves ranging between 77 to 98%, said composition consisting of MS basal culture medium, 6- benzyl-aminopurine (BAP) about 2.0 mg/L, Naphthalene acetic acid (NAA) about 1.0 mg/L, and Gibberellic acid (GA₃) ranging between 15 to 20 mg/L.

32. (Withdrawn) Compositions as claimed in claim 25 wherein, fast *in vitro* multiplication of the said medicinal plant species, with no dormancy period can make this plant commercially available in bulk.

33. (Withdrawn) Compositions as claimed in claim 25 wherein, said compositions are used for reliable and uniform germination.

34. (Withdrawn) Compositions as claimed in claim 25 wherein, said compositions are used for initiating faster germination, with only 81 days *in vitro*, to achieve the same.

35. (Withdrawn) Compositions as claimed in claim 25 wherein, said compositions is the first step towards release of the epicotyl dormancy.

36. (Withdrawn) Compositions as claimed in claim 25 wherein, said compositions can be used for all possible genotypes of said plant species to be grown *in vitro*.

37. (Withdrawn) Compositions as claimed in claim 25 wherein, said compositions can be used growing said plant species *in vitro*, in all geographical regions and all seasons.

38. (Withdrawn) Compositions as claimed in claim 25 wherein, said compositions are used for uniform and high germination rates.

39. (Withdrawn) Compositions as claimed in claim 25 wherein, said fast *in vitro* multiplication of the said medicinal plant species, is used for conservation of this endangered plant species.

40. (Withdrawn) Compositions as claimed in claim 25 wherein, said fast *in vitro* multiplication of the said plant species for wider utilization of its medicinal properties.

41. (Currently Amended) A method for ~~extraordinarily~~ fast and synchronized *in vitro* induction of germination in *Polygonatum cirrhifolium* Royle, said method comprising:

- a. obtaining the ~~sterilized~~ seeds from plant *Polygonatum cirrhifolium* Royle, wherein the seeds are sterilized,
- b. placing the sterilized seeds in sterile disposable plastic Petri plates (10 x 2 cm) containing semi-solid culture medium with 7.5% agar,
- c. incubating the ~~parafilm-sealed~~ petri dishes at temperature ranging between 20

to 24°C and at a relative humidity (RH) ranging between 50 to 60%, wherein the petri dishes are parafilm sealed,

~~d. recording the emergence of hypocotyl in the said seeds at an interval of three days;~~

~~e. adjusting pH of the medium to 5.8 with 1N NaOH or 1N HCl,~~

~~f. sterilizing the medium for 20 minutes at 121°C and 15 lb. psi pressure,~~

~~g. dispensing the medium into petri dishes as 30 ml aliquots,~~

~~h. incorporating GA₃ into the medium after filter sterilization using 0.22 µm pore size filter to cooled autoclaved medium;~~

~~i. transferring said seeds with emerged hypocotyl under aseptic conditions using Laminar Air Flow, to first medium culture consisting of comprising MS Murashige and Skoog's (MS) basal culture medium, and Gibberellic acid (GA₃) present in an amount ranging between 10 to 100 mg/L ,~~

~~j. e. incubating the one set of said first medium culture at 30°C under continuous continuous dark conditions (table 1),~~

~~k. recording the germination percentage and duration of germination of the said seeds under the first medium culture,~~

~~l. f. incubating the a second set of said first medium culture under diurnal temperature regime of 30/20°C, continuous and continuous dark conditions (table 2), and~~

~~m. recording the germination percentage and duration of germination of the said seeds under second set of first medium culture,~~

~~n. g. transferring the said germinating seeds to a third set of first medium culture~~

at 20°C under 16 hours photoperiod in a growth chamber (table 3),

~~o. recording differentiation of epicotyl with emergent, coleoptile and radicle as germination synchronization in third set of first culture medium on daily basis,~~

wherein said first medium culture has a pH adjusted to 5.8 with 1N NaOH or 1N HCl, is sterilized for 20 minutes at 121°C and 15 lb. psi pressure, comprises GA₃ incorporated into said medium after filter sterilization using 0.22 µm pore size filter to cooled autoclaved medium and dispensed into petri dishes as 30 ml aliquots.

42. (Currently Amended) A ~~The method as claimed in~~ of claim 41 wherein, ~~first medium composition useful for initiating fast germination of said plant species with percentage germination ranging between 65 to 98% of the seeds are germinated, and duration period to achieve the same ranging within a period of between 7 to 53 days, and also useful for initiating fast wherein between 78 to 100% of seeds release of epicotyl, coleoptile and radicle in percentage seeds ranging between 78 to 100, with duration period for synchronization of the same ranging within between 11 to 18 days.~~

43. (Currently Amended) A ~~The method as claimed in~~ of claim 41 wherein, ~~first medium composition is used for fast germination, with percentage induction of germination of about 98% of the seed are germinated within is achieved in time duration ranging between 7 to 23 days, said composition consisting of MS basal culture medium and about 50 mg/L Gibberellic acid (GA₃) comprises about 50mg/L of GA₃.~~

44. (Currently Amended) A ~~The method as claimed in~~ of claim 41 wherein, ~~first medium composition is used for breaking epicotyl dormancy, with about 100% of the seeds release of the epicotyl, coleoptile and radicle, and to achieve the same in time duration ranging~~

between 11 to 14 days, said composition ~~consisting of MS basal culture medium and gibberellic acid (GA₃)~~ ranging comprising GA₃ in an amount between 50 to 100 mg/L.

45. (Currently Amended) A ~~The method as claimed in~~ of claim 41 wherein, ~~said compositions are used for initiating faster germination of seeds occurs *in vitro* within,~~ with only 81 days ~~*in vitro*, to achieve the same.~~

46. (Currently Amended) A ~~The method as claimed in~~ of claim 41 wherein, ~~said first medium further comprises~~ plant hormones ~~are selected a from~~ the group ~~comprising~~ consisting of ~~gibberellic acid (GA₃),~~ alpha- naphthalene acetic acid (NAA), and 6-benzyl-aminopurine (BAP).

47. (Currently Amended) A ~~The method as claimed in~~ of claim 41 wherein, ~~Murashige and Skoog's (MS) MS basal culture medium is consisting of~~ comprises 2.2g/L NH₄NO₃, 2.0g/L KNO₃, 0.44 g/ l CaCl₂. 2H₂O, 0.37 g/L MgSO₄. 7H₂O, 0.17g/L KH₂PO₄, 37.25 mg/L Na₂ EDTA, 27.8 mg/L FeSO₄.7H₂O, 0.83 mg/L KI, 6.2 mg/L H₃ BO₃, 22.3 mg/L MnSO₄. 4H₂O, 8.6 mg/L ZnSO₄. 7H₂O, 0.25 mg/L Na₂ MoO₄. 2H₂O, 0.025 mg/L CuSO₄. 5H₂O, 0.025 mg/L CoCl₂.6H₂O, supplemented with 250 mg/L ~~myo-inositol~~ myo-inositol, 0.5mg/l nicotinic acid, 0.5 mg/L pyridoxine HCl, 3 mg/L thiamine HCl, 2 mg/L glycine, and 30g/L sucrose.

48. (Currently Amended) A ~~The method as claimed in~~ of claim 41 wherein, ~~said seeds are washed~~ further comprising washing the seeds thoroughly for 2 hours under tap water with 1-2 drops of ~~Tween-20~~ Tween-20™ and ~~rinsed~~ rinsing with distilled water.

49. (Currently Amended) A ~~The method as claimed in~~ of claim 41 ~~42~~ wherein, ~~the rinsed~~ seeds are sterilized with 0.1% mercuric chloride (HgCl₂).

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57. (Currently Amended) A method for ~~extraordinarily faster~~ inducing release of epicotyl dormancy in *Polygonatum cirrhifolium* Royle ~~using second medium composition consisting of comprising MS basal culture medium, 6-benzyl aminopurine (BAP) ranging between 3 to 6 mg/L, and Naphthalene acetic acid (NAA) ranging between 0.5 to 1.0 mg/L, said method comprising:~~

a. preparing a medium composition comprising MS basal culture medium, 6-benzyl-aminopurine (BAP) present in an amount ranging between 3 to 6 mg/L, and naphthalene acetic acid (NAA) present in an amount ranging between 0.5 to 1.0 mg/L,

~~incubating the parafilm-sealed petri dishes at temperature ranging between 20 to 24°C and relative humidity (RH) ranging between 50 to 60%,~~

~~b. transferring secondary explants consisting of epicotyl with emergent coleoptile and radicle obtained, from said germinating seeds, under aseptic conditions, into the said dishes, using Laminar Air Flow,~~

e. b. adjusting pH of the medium to 5.8 with 1N NaOH or 1N HCl,

d. c. sterilizing the medium for 20 minutes at 121°C and 15 lb. psi pressure,

e. ~~adding BAP and NAA to the above medium to form second medium culture,~~

~~said composition consisting of MS basal culture medium, 6-benzyl-aminopurine (BAP) ranging between 3 to 6 mg/L, and Naphthalene acetic acid (NAA) ranging between 0.5 to 1.0 mg/L (table 4);~~

~~f. d. dispensing the medium into petri dishes as 30 ml aliquots,~~

~~e. transferring seedlings consisting of epicotyl with emergent coleoptile and radicle obtained, from said germinating seeds, under aseptic conditions, into the said dishes, using Laminar Air Flow, and~~

~~g. f. incubating the said culture dishes at 20°C under 16 hr photoperiod with light intensity of 2000 lux provided by cool, white fluorescent tubes of 40 watts, and~~

~~h. recording the optimal response in release of first foliage leaf from the explant.~~

58. (Currently Amended) [[A]] ~~The method as claimed in of claim 57 wherein, second medium composition useful for initiating fast release of first foliage leaf from epicotyl dormant said explants, with percentage secondary explants producing leaves ranging between 65 to 86% of the seedlings produce leaves, mean number of leaves per said secondary explant seedlings exhibiting a mean number of leaves ranging between 2.7 to 4, and a mean leaf length ranging between 3 to 6 cms.~~

59. (Currently Amended) [[A]] ~~The method as claimed in of claim 57 wherein, second medium composition is used for initiating fast release of first foliage leaf from said explants, with about 86% of the secondary explants producing seedlings produce leaves, said seedlings exhibit a mean number of leaves of about 3.4 mean number of leaves per secondary explant seedling, and a mean leaf length of about 6 cms mean leaf length, wherein said composition consisting of comprises MS basal culture medium, 6-benzyl-aminopurine (BAP) ranging~~

~~between 3 to 6 mg/L, and Naphthalene acetic acid (NAA)~~ NAA present in an amount of about 1.0 mg/L.

60. (Currently Amended) [[A]] ~~The method as claimed in~~ of claim 57 wherein[[,]] said composition further comprises plant hormones ~~are~~ selected [[a]] from the group ~~comprising~~ consisting of gibberellic acid (GA₃), alpha- naphthalene acetic acid (NAA), and 6-benzyl-aminopurine (BAP).

61. (Currently Amended) [[A]] ~~The method as claimed in~~ of claim 57 wherein[[,]] ~~Murashige and Skoog's (MS)~~ MS basal culture medium ~~is consisting of~~ comprises 2.2g/L NH₄NO₃, 2.0g/L KNO₃, 0.44 g/ l CaCl₂. 2H₂O, 0.37 g/L MgSO₄. 7H₂O, 0.17g/L KH₂PO₄, 37.25 mg/L Na₂ EDTA, 27.8 mg/L FeSO₄.7H₂O, 0.83 mg/L KI, 6.2 mg/L H₃ BO₃, 22.3 mg/L MnSO₄. 4H₂O, 8.6 mg/L ZnSO₄. 7H₂O, 0.25 mg/L Na₂ MoO₄. 2H₂O, 0.025 mg/L CuSO₄. 5H₂O, 0.025 mg/L CoCl₂.6H₂O, supplemented with 250 mg/L myo-inosital, 0.5mg/l nicotinic acid, 0.5 mg/L pyridoxine HCl, 3 mg/L thiamine HCl, 2 mg/L glycine, and 30g/L sucrose.

62. (Currently Amended) [[A]] ~~The method as claimed in~~ of claim 57 ~~wherein, said seeds are washed~~ , further comprising washing seeds used to produce the seedlings thoroughly for 2 hours under tap water with 1-2 drops of ~~Tween-20~~ Tween-20 TM and ~~rinsed~~ rinsing the seeds with distilled water.

63. Currently Amended) [[A]] ~~The method as claimed in~~ of claim 57 62, wherein[[,]] said rinsed seeds are sterilized with 0.1% mercuric chloride (HgCl₂).

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69. (Cancel)

70. (Cancel)

71. (Currently Amended) A method for ~~extraordinarily faster~~ inducing release of epicotyl dormancy from differentiated de novo axillary bud and release of foliage leaves in *Polygonatum cirrhifolium* Royle, ~~using third medium composition consisting of MS basal culture medium, 6-benzyl-aminopurine (BAP) about 2.0 mg/L, Naphthalene acetic acid (NAA) about 1.0 mg/L, and Gibberellic acid (GA₃) ranging between 5 to 20 mg/L, said method comprising:~~

a. preparing a medium composition comprising MS basal culture medium, 6-benzyl-aminopurine (BAP) present in an amount about 2.0 mg/L, naphthalene acetic acid (NAA) present in an amount about 1.0 mg/L, and gibberellic acid (GA₃) present in an amount ranging between 5 to 20 mg/L, wherein

~~incorporating GA₃ into the fresh MS basal culture medium with NAA and BAP, after filter sterilization using 0.22 µm pore size filter to cooled autoclaved medium, to form third medium culture, said composition consisting,~~

GA₃ is incorporated into MS basal culture medium containing NAA and BAP after filter sterilization using a 0.22mm pore size filter to cooled autoclaved medium,

b. ~~transferring fresh secondary explants consisting of~~ seedlings comprising epicotyl with emergent coleoptile and radicle obtained from said germinating seeds ~~explant incubated~~ under aseptic conditions using Laminar Air Flow, ~~to third culture medium,~~

c. incubating the said ~~culture~~ seedlings at about 20°C under duration ranging

between 10 to 6 hr photoperiod, with light intensity of about 2000 lux ~~provided by cool,~~

d. maintaining the ~~incubated cultures~~ seedlings at 50-60% [[RH]] relative humidity,

e. subculturing the ~~said culture~~ seedlings after every four weeks ~~on the third~~
~~medium formulations as mentioned above~~ onto fresh medium compositions

f. ~~recording the de novo axillary bud differentiation and release of foliage leaves~~
~~from them, and~~

g. ~~compiling the final data after 16 weeks of culture on the basis of periodical~~
~~observations.~~

72. (Currently Amended) [[A]] The method as claimed in of claim 71 wherein, third
~~medium composition useful for initiating fast release of axillary buds from epicotyl dormant said~~
~~explants, with secondary explants producing axillary buds ranging between 70 to 100% of the~~
seedlings produce axillary buds, said seedlings exhibiting a mean number of axillary buds per
~~secondary explant ranging between 6 to 12, and wherein axillary buds producing leaves ranging~~
between 45 to 98% of said axillary buds produce leaves.

73. (Currently Amended) [[A]] The method as claimed in of claim 71 wherein, third
~~medium composition used for initiating fast release of axillary buds from said explants, with~~
~~about 100%[[,]] of the secondary explants seedlings producing axillary buds, number of axillary~~
~~buds per secondary explant seedling ranging between 9 to 12, and wherein axillary buds~~
~~producing leaves ranging between 77 to 98% of said axillary buds produce leaves, said~~
~~composition consisting of MS basal culture medium, 6-benzyl aminopurine (BAP) about 2.0~~
~~mg/L, Naphthalene acetic acid (NAA) about 1.0 mg/L, and Gibberellic acid (GA3) comprises~~

GA₃ present in an amount ranging between 15 to 20 mg/L.

74. (Cancel)

75. (Currently Amended) [[A]] The method as claimed in of claim 71 wherein;
~~Murashige and Skeog's (MS)~~ MS basal culture medium ~~is consisting of~~ comprises 2.2g/L
NH₄NO₃, 2.0g/L KNO₃, 0.44 g/ l CaCl₂. 2H₂O, 0.37 g/L MgSO₄. 7H₂O, 0.17g/L KH₂PO₄, 37.25
mg/L Na₂ EDTA, 27.8 mg/L FeSO₄.7H₂O, 0.83 mg/L KI, 6.2 mg/L H₃ BO₃, 22.3 mg/L MnSO₄.
4H₂O, 8.6 mg/L ZnSO₄. 7H₂O, 0.25 mg/L Na₂ MoO₄. 2H₂O, 0.025 mg/L CuSO₄. 5H₂O, 0.025
mg/L CoCl₂.6H₂O, supplemented with 250 mg/L myo-inositol, 0.5mg/l nicotinic acid, 0.5 mg/L
pyridoxine HCl, 3 mg/L thiamine HCl, 2 mg/L glycine, and 30g/L sucrose.

76. (Currently Amended) [[A]] The method as claimed in of claim 71 wherein, said seeds
~~are washed~~ , further comprising washing seeds used to produce the seedlings thoroughly for 2
hours under tap water with 1-2 drops of Tween-20 Tween-20 TM and rinsed rinsing the seeds
with distilled water.

77. (Currently Amended) [[A]] The method as claimed in of claim 71 76 wherein[[,]] said
rinsed seeds are sterilized with 0.1% mercuric chloride (HgCl₂).

78. (Cancel)

79. (Cancel)

80. (Cancel)

81. (Cancel)

82. (Cancel)

83. (Cancel)

84. (Cancel)